Original Research Article

Genetic variability and association analysis of Lisianthus (*Eustoma grandiflorum* (Raf) Shinn) advanced lines under mid hill conditions of Himachal Pradesh, India

Abstract: The success of a good breeding programme usually depends upon the genetic variability present in the breeding materials; however, the amount, kind and magnitude of variability as well as genetic relationship of traits in lisianthus are not efficiently exploited yet. The present investigation was designed to assess the extent of variability, genetic advance, heritability and interrelation of different traits of 19 lisianthus genotypes at ICAR-IARI Regional Station, Katrain, Kullu, HP during 2019–2021. The mean performance of different genotypes exhibited considerable range together with large value for most of the characters. The trend of variability at genotypic level was similar to that of at phenotypic for some of the characters. The path analysis clearly indicated that total number of flowers per plant was directly associated with plant height and number of shoots per plant. It is imperative that these traits should be prioritized while improving number of flowers per plant in lisianthus. The cluster analysis revealed existence of diversity among the evaluated genotypes. The first principal component analysis (PCA) score explained 33.798% of the total variation mainly associated to genotype and flower yield. The PCA biplot was effective in showing the genetic distance among the genotypes and their discrimination based on key traits of importance in lisianthus. Genotypes Ktlis-1, Ktlis-17, Ktlis-5, Ktlis-9 and Ktlis-7 were superior among the tested genotypes therefore, these could be exploited in lisianthus breeding to improve flower yield. Hence, the characters showing high heritability along with high genetic gain should be given due attention in the development of desirable genotypes through simple selection. Further, Genotypes from different clusters identified for specific characters may be used as parent for lisianthus breeding programme with an objective to improve the specific traits.

Key word: *Lisianthus, PCA, path coefficient analysis, clustering, genetic variability*

1. Introduction

The Lisianthus (*Eustoma grandiflorum* (Raf.) Shinn.) is an herbaceous ornamental plant belongs to the Gentianaceae family and commonly known as Texas Blue Bell'; 'Tulip gentian' and 'Prairie Gentian'. This genus was originating from northern Mexico and the

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southern United States (Kuronuma *et al*. 2018). It is commonly known as 'Texas Blue Bell'; 'Tulip gentian' and 'Prairie Gentian'. In the last decade, it has emerged as one of the fastest growing segment of new flower category worldwide and demand has increased due to its ornamental characteristics, including a long vase life, wide range of colours (from purple to lavender, and from pink to white), as well as high crop profitability. It is cultivated as a cut flower or as flowering pot plants (Anderson, 2006). Production of lisianthus has increased dramatically in recent years, spurred by the development of excellent cultivars in a wide range of colours, both in single and double forms (Reid, 2000). Its flowers are widely used for making bridal bouquets and many other special flower arrangements. Lisianthus has been recently introduced in India as a new specialty cut flower. *Eustoma grandiflorum* is of large flowers and restricted distribution conforms to the genus Eustoma together with *E. exaltatum*, which has small and more widespread flowers (Turner, 2014). *Eustoma grandiflorum* presents adaptation difficulties at temperatures above 25°C (Roni *et al*., 2016) which has led to studies on genetic improvement programs. The genetic variation created is useful because it helps population to survive and change over time.

In temperate regions of Himachal Pradesh, lisianthus for cut flowers is generally sown during the winter (November-December); when temperatures are lower than optimal. The seedlings are transplanted under protected conditions during April-May. The unfavourable conditions enhance the expression of the genetic variation among genotypes of lisianthus with respect to their growth features. These unfavourable conditions enhance the expression of the genetic variation among cultivars and genotypes of lisianthus, with respect to their growth features. The success of good breeding programme usually depends upon the genetic variability present in the breeding materials, so assessment of genetic variability in the base population should have to be prior action in breeding programme. Information on the relative magnitude of different sources of variation among different genotypes for several traits helps in measurement of their range of genetic diversity. The genetically diverse genotypes are likely to produce heterotic effect and superior segregate when incorporated in hybridization to hasten crop improvement programme. Thus, knowledge on genetic variability, heritability and genetic advance is essential for a breeder to choose and for efficient utilization of better genotypes for crop improvement programs. Cluster and principal component analysis (PCA) are useful tools for the determination of genetic relationship among genotypes in crop improvement. This is due to the fact that they group genetically similar genotypes together and create a scatter plot of genotypes with the geometrical distances among them reflecting their genetic distances with minimum distortion, respectively (Pereira et al., 2015). Therefore,

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the present study aimed to assess and quantifies the level of genetic variability present among tested lisianthus lines and to determine the significance of various economic traits.

2. Materials and methods

2.1. Plant materials

Nineteen lisianthus genotypes like Ktlis-11, Ktlis-12, Ktlis-8, Ktlis-14, Ktlis-19, Ktlis-15, Ktlis-17, Ktlis-1, Ktlis-5, Ktlis-18, Ktlis-16, Ktlis-2, Ktlis-6, Ktlis-20, Ktlis-21, Ktlis-13, Ktlis-7, Ktlis-10 and Ktlis-9 developed using reverse breeding. These genotypes were evaluated under protected conditions at the station.

2.2. Experimental site

The genotypes were evaluated at office farm of ICAR-Indian Agricultural Research Institute Regional Station, Katrain, Kullu, Himachal Pradesh during 2019-2021. The farm is situated at 32.12°N latitude and 77.13°E longitude, at an altitude of 1460 m above mean sea level and it receives an average annual rainfall and snowfall of 110–120 cm and 120–150 cm, respectively.

2.3. Seed sowing and transplanting

The seeds of nineteen lisianthus genotypes were sown during December in a conventional germination media containing leaf mould, coco peat, perlite and vermiculite in equal proportions. Seed was placed on the surface of growing media and was not covered. The seedlings after 30-35 days of sowing were fertigated with water soluble fertilizer (NPK: 19:19:19) @ 1 g/l and Calcium nitrate @1.5 g/l at weekly intervals because the initial seedling development is very slow and thus fertigation is factor for proper vegetative growth. Seedlings having five pairs of true leaves were transplanted under protected structures at spacing15 cm (plant to plant) and 20 cm (from row to row) in a well prepared and sterilized bed after 80-90 days of seed sowing.

2.4. Data collection

Five plants per genotypes were tagged randomly for recording plant-based characters. Morphological characterization was carried out based on guide TG / 197/1. Guidelines for the execution of the examination of the distinction, homogeneity and stability of Eustoma (UPOV, 2002), established by International Union for Protection of New Varieties of Plants (UPOV). Leaf area was estimated by linear measurements using 'K' factor as 0.70 as suggested by Anitha *et al*. (2016). The flower colour observations were taken as per the guide of the Royal Horticultural Society (RHS) as marked by the guide TG / 197/1 (UPOV, 2002) of Eustoma.

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2.5. Data analysis

The data were subjected to analysis of variance as per the procedure described by Gomez and Gomez (1983) and as per the formulae described by Panse and Sukhatme (1967) using OPSTAT software (Sheoran *et al.* 1998). The Pearson's correlation coefficient, principal component analysis (PCA) and UPGMA dendrogram based on average linkage (between groups) was done through SPSS 16.0 software. Heritability and genetic advance were calculated according to Allard (1960) and genetic gain was estimated as per the method given by Johanson *et al*. (1955). Multivariate analysis was done utilizing Mahalanobis D^2 statistics and genotypes were grouped into five different clusters following Tochers method as described by Rao (1952). Cluster means were calculated for individual character on the basis of mean performance of the genotypes included within the cluster.

3. Results and Discussion

3.1. Mean performance of genotypes

Genetic variability is the basic need for a plant breeder to initiate any breeding programme. Among the different floricultural traits under study, wide range was observed for the plant height (43.50-82.98 cm), number of flowers/stem (5.08-10.0), number of petals per flowers (5-29.33), bud length (3.92-5.18 cm), flower diameter (4.63-7.99 cm) and leaf area (15.84-31.57 cm^2) (Table 1). The wide variations with respect to different growth characters might be attributed to inherent genetic characters of the genotypes as reported earlier by Harbugh *et al*. (2000), Anitha *et al*. (2013) and Uddin *et al*. (2013) in lisianthus. Ecker *et al*. (1994) had reported that stem length at harvest is a combined result of the rate of stem elongation and the period from planting to flowering.

3.2. Parameters of variability

3.2.1. Coefficient of variability

Estimation of genotypic and phenotypic coefficients of the genetic variability in breeding material is essential for a successful plant breeding programme. Understanding the magnitude of variability in crop species is pivotal since it provides the foundation for selection. The estimates of phenotypic and genotypic coefficient of variability gave a clear picture of amount of variations present in the available germplasm (Table 2). For all the characters studied, the magnitude of phenotypic coefficient of variation (PCV) is higher than genotypic coefficient variability (GCV). But, for three traits viz. plant height, flower diameter and number of petals per flower, the difference between PCV and GCV is very meagre. It means that ~~that~~ these traits are not much influenced by environmental factors. Hence,

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selection based on the phenotypic performance of above-mentioned traits will be more reliable and effective. The genotypic coefficient of variability (GCV) ~~were~~ was recorded higher for number of petals per flower, leaf area, number of flowers per stem and plant height. The high GCV indicates the presence of exploitable genetic variability for the traits, which can facilitate selection. Whereas, low GCV ~~were~~ was recorded for bud length, number of shoots per plant and flower diameter. Similar results were noticed by Anitha *et al*. (2013) in lisianthus, Ravikumar and Patil (2003) in china aster and Namita *et al*. (2008) in French marigold.

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3.2.2. Heritability and Genetic gain

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The magnitude of heritability estimates gives an insight into the extent of genetic control to express a particular trait and phenotypic reliability in predicting its breeding value (Ndukanba *et al*. 2015). High heritability indicates less environmental influence in the observed variation. Broad-sense heritability (h^2bs) only indicates whether or not there is sufficient genetic variation in a population, which implies whether or not a population will respond to selection pressure. The estimates of heritability (h^2bs) were found high for the traits viz., plant height, number of petals per flower and flower diameter. It was moderate for leaf area, number of flowers per stem and bud length. The estimates of heritability were low for number of shoots per plant and total number of flowers per plant. The results are in accordance with the findings of Anitha *et al*. (2013) in lisianthus. These results indicate that there is a considerable genetic variation present in these traits to warrant selection for better accessions. These traits can therefore be given special attention for selections aimed at lisianthus breeding.

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To access a more effective trait selection, high heritability accompanied by high/moderate genetic gain is more useful than heritability alone. In the present study, high heritability estimates coupled with high genetic gain were observed for plant height and number of petals per flower, indicated that these characters are under additive gene effects and these are reliable for most effective condition for selection (Panse, 1957). The obtained results are in close conformity with findings of Anitha *et al*. (2013) in lisianthus, Deepti Singh and Kumar (2008) in marigold, Shiekh and John (2005) in iris for plant height. The high genetic gain for plant height and number of petals per flower suggest that the variation in these traits was mainly genetic with less environmental influence coupled with the prevalence of additive gene action in their inheritance (Tazeen *et al*.2009).

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3.3. Character association and path analysis

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3.3.1. Character association

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The direction and level of relationship among different traits determine the efficiency of selection. Correlation coefficients give us information about the nature and extent of association and thus help in the selection for the improvements of traits. The results pertaining to correlation studies are presented in table 3. The estimates of genotypic correlation coefficients, in general, were higher in magnitude than the phenotypic coefficients for most of the traits indicating least influence of environmental factors in the expression of associations among these traits. The data revealed a significant positive correlation of plant height with number of flowers per stem (0.588), bud length (0.529), leaf area (0.420) and total number of flowers per plant (0.746). Number of flowers per stem also showed a significant positive correlation with number of shoots per plant (0.489), and total number of flowers per plant (0.787); number of shoots per plant shows positive correlation with total number of flowers per plant (0.975). Bud length significantly and positively correlated with flower diameter (0.670), leaf area (0.797) and total number of petals per flower (0.332). Diameter of flower showed positive and significant correlation with leaf area (0.482) and total number of petals per flower (0.697), whereas, leaf area was positively correlated with total number of petals per flower (0.605). Leaf area showed significantly negative correlation with number of flowers per stem (-0.288) and number of shoots per plant (-0.644). Similarly, total number of flowers per plant shows significant negative correlation with leaf area (-0.452), indicating that the direct selection for these traits may not be useful. These results are in line with the findings of Dhiman *et al*. (2020) and Ecker *et al*. (1994) in lisianthus. Ecker *et al*. (1994) reported that flower initiation in lisianthus is triggered by an independent genetic factor which can be activated only after stem elongations.

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3.3.2. Path analysis

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The correlation analysis may not provide a clear picture of the importance of each secondary trait in determining the yield. The path coefficient analysis allows separation of direct and indirect effects by partitioning the correlation coefficients allowing the estimates of contribution of each component traits. Path analysis was carried out by taking total number of flowers per plant as dependent variables and the remaining traits as independent variables. The direct and indirect effects of various traits on total number of flowers per plant were depicted in table 4. The results revealed that plant height showed highest positive direct effect (r=1.369) on total number of flowers per plant followed by number of shoots per plant (r=0.782) and number of petals per flower (r=0.318). The positive indirect effect of plant height was found on total number of flowers per plant via number of shoots per plant

(r=0.133) and number of petals per flower (r=0.073), whereas, number of flowers per stem showed positive indirect effect on total number of flowers per plant via plant height (r= 0.804), number of shoots per plant (r=0.367), bud length (r=0.014), flower diameter (r=0.050), leaf area (r=0.135) and number of petals per flower (r=0.032). Number of shoots per plant reflected indirect effect on total number of flowers per plant via plant height (r=0.233), leaf area (r=0.302) and number of petals per flower (r=0.044). Bud length affected the total number of flowers per plant via plant height (r=0.725), number of flowers per stem (r=0.022), number of shoots per plant (r=0.132) and number of petals per flower (r=0.105). While, Flower diameter revealed significant positive indirect effect on total number of flowers per plant via plant height (r=0.287), number of flowers per stem (r=0.148), number of shoots per plant (r=0.028) and number of petals per flower (r=0.221). Leaf area exhibited positive indirect effect on total number of flowers per plant via plant height (r=0.575), number of flowers per stem (r=0.183) and number of petals per flower (r=0.192). On the other hand, number of petals per flower affected the total number of flowers per plant via plant height (r=0.318) and number of shoots per plant (r=0.109). The findings of these results suggest that there is enough scope of improvement of these traits through selection. These results are in accordance with the findings of Dhiman *et al*. (2015) in alstroemeria.

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3.4. Genetic Divergence studies

3.4.1. Cluster composition

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After computing D^2 values for all the possible pairs, 19 genotypes were grouped into five clusters, which indicated a wider genetic diversity (Table 5). Cluster III and IV accommodated maximum of genotypes (6 each), followed by cluster II with five genotypes. While the cluster I and V accommodated only one genotype each. The grouping of genotypes indicated that geographical origin had no influence on clustering pattern. Moreover, this is an indication that geographical diversity is not a measure of genotypic diversity. Average intra and inter cluster distance for 19 lisianthus genotypes were presented in table 5. Cluster III exhibited maximum intra-cluster distance (D^2= 305.298) followed by cluster II (D^2=221.835) and cluster IV with minimum intra-cluster distance (D^2=205.299). Cluster I and V exhibited zero distance as they possess single genotype only. Inter-cluster distance depict that I and cluster V had maximum divergence (D^2=47.678). The lowest inter-cluster distance (D^2=10.769) was recorded between cluster III and cluster IV indicating existence of closer proximity between these clusters. The diverse genotypes characterized by maximum inter-cluster distance will differ in phenotypic performance and therefore, selection of divergent

parents should be based on these cluster distances to obtain favourable hybrids and transgressive segregants in lisianthus.

3.4.2. Cluster means

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For any crop improvement programme, inter-crossing among genotypes with outstanding mean performance was suggested by Roy and Sharma (1996). The cluster means of the different traits are presented in table 6. Moreover, for getting the reliable conformity on the basis of cluster means, cluster-IV exhibited maximum number of flowers per plant (24.67), flower diameter (6.91), number of shoots per plant (4.35) and number of flowers per stem (7.94). Cluster- I gave maximum mean values for plant height (82.98), bud length (4.99) and leaf area (37.05) and minimum mean values for number of shoots per plant (3.42). Cluster-V recorded minimum mean value for bud length (4.37) and flower diameter (5.87). The genotypes having broad genetic base and desirable traits can be involved in crosses which would lead to transmission of genetic gain for various putative traits including cut flower production for practical utility. Hence, hybridization between genotypes accounted wider genetic variance likely to be effective for developing promising divergent heterotic cross combination. Therefore, lisianthus genotypes has to be earnestly exploited spatially and temporarily in breeding programme.

3.4.3. Principal Component analysis

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The principal component analysis (PCA) is important for the reflection of the highest contributor to the total variation at each axis of differentiation. The Eigen values from PCA are used for determination of how many factors to retain. In the present investigation, only the first three principal components with Eigen values greater than one based on methods proposed by Kaiser (1960) were used and cumulatively they explained 74.170% variability (Table 7). The first principal component (PC1) had highest positive value for plant height (0.748), flower diameter (0.660), bud length (0.652) and total number of petals per flower (0.625). The second principal component (PC2) had highest positive values for number of flowers per plant (0.749) and number of flowers per stem (0.723), while third principal component (PC3) exhibited number of shoots per plant (0.621) only. Further, loading of different characters based on two principal components indicated that plant height, number of flowers per stem, number of shoots per plant and total number of flowers per plant were loaded more positively on two ~~axes,~~axis while bud length, flower diameter, leaf area and number of petals per flower were loaded negatively on Y-axis (Fig.1). The dendrogram constructed using average linkage hierarchial cluster analysis classified nineteen genotypes

into four major group's viz., A, B, C and D (Fig.2). The group 'A' accommodate only one genotype i.e. Ktlis-19, group 'B' accommodate four genotypes (Ktlis-12, Ktlis-18, Ktlis-2 and Ktlis-16), group 'C' eight genotypes (Ktlis-14, Ktlis-13, Ktlis-20, Ktlis-7, Ktlis-5, Ktlis-11, Ktlis-21 and Ktlis-1) and group 'D' accommodated six (Ktlis-8, Ktlis-6, Ktlis-15, Ktlis17, Ktlis19 and Ktlis-10) genotypes, respectively. These results are in line with the findings of Dhiman et al. (2020), Ahmad et al. (2017) in lisianthus and Sangeeta et al. (2019) in lilium.

Conclusion

The estimates of genotypes mean exhibited considerable range together with large value for most of the characters. The trend of variability at genotypic level was similar to that of at phenotypic for some of the characters. The path analysis clearly indicated that total number of flowers per plant was directly associated with plant height and number of shoots per plant. It is imperative that these traits should be prioritized when improving number of flowers per plant in lisianthus. The cluster analysis revealed existence of diversity among the evaluated genotypes. The first principal component analysis score explained 33.798% of the total variation mainly associated to genotype and flower yield. The PCA biplot was effective in showing the genetic distance among the genotypes and their discrimination based on key traits of importance in lisianthus. Genotypes Ktlis-1, Ktlis-17, Ktlis-5, Ktlis-9 and Ktlis-7 were superior among the tested genotypes therefore, these could be exploited in lisianthus breeding to improve flower yield. Hence, the characters showing high heritability along with high genetic gain should be given due attention in the development of desirable genotypes through simple selection. Genotypes from different clusters identified for specific characters may be used as parent for breeding programme with an objective to improve the specific traits.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Mean performance of different lisianthus genotypes for growth and flowering traits

S.No	Genotypes	Plant height (cm)	No. of flowers /stem	No. of shoots/ plant	Bud length (cm)	Flower diameter (cm)	Leaf area cm^2)	Total number of flowers /plant	No. of petals /flower
1.	Ktlis-11	64.85	7.08	4.25	5.18	7.32	26.40	17.17	29.33
2.	Ktlis-12	46.21	5.08	3.50	4.73	6.70	24.22	13.50	24.25
3.	Ktlis-8	59.79	7.25	4.25	4.79	6.91	19.38	20.58	17.33
4.	Ktlis-14	64.64	7.50	3.67	4.78	6.51	24.24	23.00	21.33
5.	Ktlis-19	43.50	5.92	4.00	4.37	5.87	17.66	19.92	5.00
6.	Ktlis-15	63.36	6.75	3.67	4.43	6.70	23.27	14.50	17.58
7.	Ktlis-17	65.75	10.00	3.25	4.62	5.88	23.78	18.92	17.00
8.	Ktlis-1	82.98	6.83	3.42	4.99	6.89	37.05	24.17	22.83
9.	Ktlis-5	69.00	9.17	3.83	4.83	7.18	20.25	27.00	26.33
10.	Ktlis-18	52.10	6.00	3.58	4.14	5.72	18.26	18.75	22.75
11.	Ktlis-16	52.03	5.67	3.00	4.95	7.16	26.69	17.33	22.00
12.	Ktlis-2	55.10	6.17	3.58	3.92	5.73	23.27	15.33	21.92
13.	Ktlis-6	60.51	7.17	4.25	4.46	6.02	20.65	22.75	17.17
14.	Ktlis-20	64.55	8.83	4.58	4.57	6.49	24.54	27.42	20.33
15.	Ktlis-21	59.50	5.58	4.33	4.93	7.99	31.37	18.67	25.17
16.	Ktlis-13	67.13	6.00	4.42	4.84	7.83	23.94	23.58	24.00
17.	Ktlis-7	65.78	9.08	5.33	4.47	6.16	22.33	29.83	23.00
18.	Ktlis-10	65.50	6.50	4.08	4.97	4.63	19.96	21.33	5.00
19.	Ktlis-9	70.98	7.50	3.75	4.52	7.01	15.84	21.67	16.50
	CD (P=5%)	**6.43**	**2.15**	**NS**	**0.62**	**0.81**	**7.71**	**NS**	**4.05**
	SE(m)	2.23	0.75	0.46	0.22	0.28	2.68	3.40	1.41
	SE(d)	3.16	1.06	0.65	0.30	0.40	3.79	4.80	1.99
	C.V.	6.27	18.36	20.16	7.99	7.40	19.89	28.27	12.20

Table 2: Mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advances (GA) in lisianthus genotypes

Trait	Mean	Phenotypic coefficient variation (%)	Genotypic coefficient variation (%)	Heritability in broad sense (%)	Genetic advance (%)	Genetic gain means (%)
Plant height	61.75	15.69	14.38	84.05	16.77	27.16
Number of flowers/stem	7.00	25.34	15.84	39.06	1.43	20.39
Number of shoots per plant	3.63	21.49	7.47	12.06	0.21	5.34
Bud length	4.66	9.41	4.97	27.91	0.25	5.41
Flower diameter	6.56	13.82	11.68	71.40	1.33	20.32
Leaf area	23.32	26.62	17.70	44.19	5.65	24.24
Total number of flowers per plant	20.81	31.41	13.69	18.99	2.56	12.29
Number of petals per flowers	19.94	32.13	30.80	86.43	11.76	58.96

Table 3. Genotypic (g) and phenotypic (p) coefficient correlations among the traits of lisianthus

Traits		Plant height	Number of flower/stem	Number of shoots/plant	Bud length	Flower dia.	Leaf area	Total no. of flowers/plant	Total number of petals /flower
Plant height	G	0.000							
	P	0.000							
Number flower/stem	G	0.588**							
	P	0.367**							
Number of shoots/plant	G	0.170	0.489**						
	P	0.149	0.191						
Bud length	G	0.529**	-0.035	0.168					
	P	0.273*	0.034	-0.031					
Flower diameter	G	0.209	-0.234	0.035	0.670**				
	P	0.210	-0.068	0.092	0.344**				
Leaf area	G	0.420**	-0.288*	-0.644**	0.797**	0.482**			
	P	0.328*	0.093	0.082	0.265*	0.369**			
Total no. of flowers/plant	G	0.746**	0.787**	0.975**	0.058	-0.032	-0.452**		
	P	0.446**	0.527**	0.481**	0.153	0.048	0.204		
Total number of petals /flower	G	0.232	0.102	0.140	0.332*	0.697**	0.605**	0.110	0.000
	P	0.164	0.038	-0.033	0.123	0.603**	0.332*	-0.046	0.000

*Significant at 5% level, ** significant at 1% level.

Table 4: Estimates of direct and indirect effects of different traits on total number of flowers per plant in Lisianthus

Trait(s)	Plant height (cm)	No. of flowers/ stem	No. of shoots/ plant	Bud length (cm)	Flower diameter (cm)	Leaf area (cm^2)	No. of petals /flower	Total number of flowers/ plant
Plant height (cm)	**1.369**	-0.372	0.133	-0.216	-0.045	-0.196	0.073	0.746**
No. of flowers/ stem	0.804	**-0.633**	0.382	0.014	0.050	0.135	0.032	0.787**
No. of shoots/ plant	0.233	-0.309	**0.782**	-0.069	-0.008	0.302	0.044	0.975**
Bud length (cm)	0.725	0.022	0.132	**-0.406**	-0.145	-0.373	0.105	0.060
Flower diameter (cm)	0.287	0.148	0.028	-0.274	**-0.217**	-0.226	0.221	-0.033
Leaf area (cm^2)	0.575	0.183	-0.504	-0.326	-0.104	**-0.469**	0.192	-0.452**
No. of petals /flower	0.318	-0.065	0.109	-0.136	-0.151	-0.284	**0.318**	0.109

Residual effect = -0.51663

Table 5: Clustering pattern and average intra and inter cluster distance (D^2) of 19 genotypes of Lisianthus

Cluster	Number of genotypes	Genotypes	Cluster	I	II	III	IV	V
1.	1	Ktlis-1	I	**0.000**	33.284	26.492	21.747	47.678
II	5	Ktlis-12, Ktlis-18, Ktlis-16, Ktlis-2, Ktlis-21	II		**221.835**	15.058	15.493	21.988
III.	6	Ktlis-8, Ktlis-15, Ktlis-17, Ktlis-6, Ktlis-10, Ktlis-9	III			**305.298***	10.269	23.365
IV.	6	Ktlis-11, Ktlis-14, Ktlis-5, Ktlis-20, Ktlis-13, Ktlis-7	IV				**205.299**	30.535
V	1	Ktlis-19	V					**0.000**

Table 6: Cluster means of 5 clusters of 19 lisianthus genotypes grown under potential growing conditions

Cluster	Plant height	No. of flowers/ stem	No. of shoots/plant	Bud length	Flower Diameter	Leaf area	Total No. of flowers/plant	No. of petals/ flower
I.	82.98	6.83	3.42	4.99	6.89	37.05	24.17	22.83
II.	52.99	5.70	3.59	4.53	6.66	24.76	16.72	23.22
III.	64.31	7.53	3.87	4.63	6.19	20.48	19.96	15.09
IV.	65.99	7.94	4.35	4.78	6.91	23.62	24.67	24.05
V.	43.50	5.92	4.00	4.37	5.87	17.66	19.92	5.00

Table 7. Principal component analysis scores for the 8 quantitative traits assessed among the genotypes of lisianthus

Traits	Principal Component		
	Principal component-1	**Principal component-2**	**Principal component-3**
Plant height	0.748	0.309	-0.428
Number of flowers/stem	0.374	0.723	-0.210
Number of shoots/plant	0.298	0.565	0.621
Bud length	0.652	-0.277	-0.231
Flower diameter	0.660	-0.484	0.373
Leaf area	0.621	-0.508	-0.248
Total number of flowers/plant	0.526	0.749	0.089
Total number of petals/flower	0.625	-0.398	-0.360
Eigen Value	**2.704**	**2.226**	**1.004**
Percentage of variance	**33.798**	**27.824**	**12.547**
Cumulative %of variance	**33.798**	**61.623**	**74.170**

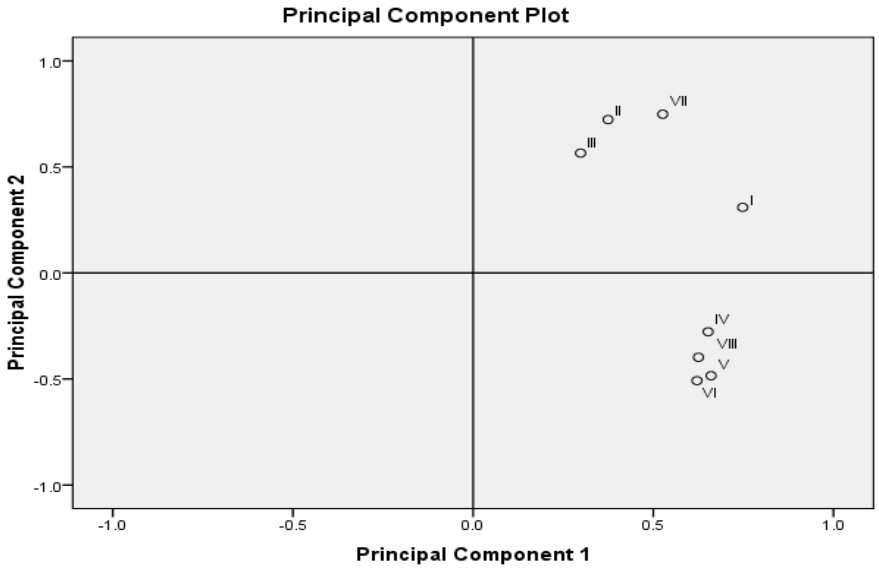

Fig. 1: Loading of different flowering traits based on first two principal components

I) Plant height, II) No. of flowers/stem, III) No. of shoots/plant, IV) Bud length, V) Flower Diameter, VI) Leaf area, VII) Total No. of flowers/plant, VIII) No. of petals/flower

Fig. 2 Dendrogram constructed using Average Linkage (Between Groups)